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Brandon, Manitoba, Canada. Aug 13-15, 1996.*

*Sponsored by: Manitoba Weed Supervisors Association and
Manitoba Agriculture.*



1996 Leafy Spurge Symposium, Victoria Inn, Brandon, Manitoba

Agenda / Posters / Sponsors

Tuesday, August 13, 1996

10 am – 1 pm *Registration*

General Session - Grand A

Moderator: Carla Pouteau, Manitoba Agriculture

1:15 pm *Opening Remarks*

- Leafy Spurge Symposium Organizing Committee
- Frank Pitura, Legislative Assistant for Department of Agriculture
- Don Kille, Acting Mayor, City of Brandon

1:30 - 2:15 *Weed Biocontrol - Lessons Learned from Leafy Spurge*

- Dr. Peter Harris, Agriculture and Agri-Food Canada, Lethbridge, Alberta.

2:15 - 2:35 *Leafy Spurge in Manitoba - The Fight to Keep Ahead*

- Carla Pouteau, Manitoba Agriculture.

2:35 - 2:55 *Biological Control of Leafy Spurge in Alberta*

- Dan Cole, Alberta Agriculture.

2:55 - 3:15 *Update on Wyoming's Leafy Spurge Program*

- Mark Ferrell, University of Wyoming.

3:15 - 3:30 *Nutritional Break - Salon 1*

3:30 - 3:50 *Niche Specificity of Insects Introduced for Leafy Spurge Control*

- Neal Spencer, USDA-ARS, Sidney, Montana.

3:50 - 4:10 *Effect of Fall-Applied Picloram and 2,4-D on *Aphthona nigriscutis* Population*

- Jeff Nelson, North Dakota State University.

4:45 *Bus Departs for Texas Scramble Golf Game and Pizza Night*

9:30 *Bus Returns to the Victoria Inn*

10:00 pm - 12:00 am *Complimentary Hospitality Suite (Victoria Inn & MWSA)
Room 117, Victoria Inn*

Wednesday, August 14, 1996

7:30 am - 8:30 am *Poster Setup - Salon 1*

General Session - Grand A

Moderator: Ray Christoff, Pembina-Manitou Weed District

8:30 am - 8:50 am *Department of National Defence's Involvement in Leafy Spurge Management To Date at Canadian Forces Base, Shilo*

- Garnet Shearer and Sherry Punak, Canadian Forces Base, Shilo, Manitoba.

8:50 - 9:10 *Phenology Maps for Leafy Spurge Biological Control Agents: Development and Application in the United States*

- Rich Hansen, USDA-APHIS-PPQ, Bozeman, Montana.

9:10 - 9:30 *Manitoba Weed Supervisors Association - Past, Present and Future?*

- Rudy Reimer, Southeast Weed District.

9:30 - 9:50 *1996 Manitoba Weed Supervisors Association and The New Utility*

- Eric Richardson, Dauphin-Ochre Weed District.

9:50 - 10:20 *Nutritional Break - Salon 1*

Moderator: Tom Tolton, Manitoba Agriculture

10:20 - 10:40 *Leafy Spurge Control on Canada's Largest Tall Grass Prairie Preserve*

- Gene Fortney, The Nature Conservancy.

10:40 - 11:10 *Imazameth is Safe on Grass; Leafy Spurge is Becoming Resistant Picloram; and Other Myths?*

- Rod Lym, North Dakota State University.

11:10 - 11:30 *Leafy Spurge Control with Sheep*

- Marcel Archambault, Manitoba Agriculture.

11:30 - 11:50 *A Simple Technique for Separating Aphthona Flea Beetles from Debris*

- David Hirsch, Jim Jeske, Terry Reule, and Doug Anderson, USDA-APHIS-PPQ, Bismark, North Dakota.

11:50 - 1:00 pm *Lunch - Grand B*

1:00 - 2:00 *Poster Session - Salon 1*

2:00 - 2:30 *Poster take down*

2:45 *Bus Departs for Field Tour*

- *Lobesia euphorbiana* release site

- GPS demonstration

- Integrated Leafy Spurge Control Project (sheep and 2,4-D)

- Herbicide demonstration

5:00 *Barbecue in Queen Elizabeth Park*

8:00 *Bus Returns to Victoria Inn*

9:00 pm *Hospitality Suite - Room 117*

Thursday, August 15, 1996

General Session - Salon 1

Moderator: Carla Pouteau, Manitoba Agriculture

- 8:30 am *Discussion Group*
- 9:30 *Business Meeting - Leafy Spurge Task Force*
- 10:30 *1996 Leafy Spurge Symposium adjourns*
- 10:45 *Bus Departs for Tour of Agriculture and Agri-Food
Canada Research
Facility*
- 11:00 *Tour of Agriculture and Agri-Food Canada Research Facility*
- 12:00 pm *Bus Returns to Victoria Inn*

Posters presented:

Aphthona Species Movement Along Railroad Right-of-Way
Katheryn Christianson, North Dakota State University.

2,4-D Reversal of DFMO-induced Inhibition of Adventitious Root Formation in Leafy Spurge (Euphorbia esula L.) In Vitro
Dave Davis, USDA-ARS, Fargo, North Dakota.

Cloning and Expression of a Cold-regulated Gene from Leafy Spurge (Euphorbia esula L.)
D. P. Horvath and P. A. Olson, USDA-ARS, Fargo, North Dakota.

Cloning and Characterization of a Genomic Clone of the Root Bud Growth-response Gene Gro7 from Leafy Spurge (Euphorbia esula L.)
D. P. Horvath, USDA-ARS, Fargo, North Dakota.

Differential Tolerance Toward Oxidative Stress Between Immature and Mature Leafy Spurge Leaves - An Evolutionary Mechanism for Surviving Adverse Environmental Conditions
R. H. Shimabukuro and B. Hoffer, USDA-ARS,
Fargo, North Dakota.

Reproductive Status of Established Black Dot Flea Beetle (Aphthona nigriscutis) Populations in South and North Dakota
Mark Brinkman, South Dakota State University.

Biological Control of Leafy Spurge with Aphthona nigriscutis
Kim Stromme, Alberta Agriculture.

Biological Weed Control in Manitoba
Manitoba Weed Supervisors Association.

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Weed biocontrol: Lessons learned from leafy spurge

PETER HARRIS

Agriculture & Agri-Food Canada Research Center, Lethbridge, Alberta.

Leafy spurge has been a difficult target for biocontrol. The problems are outlined and lessons learned in solving them are discussed. These are: 1) Start a project by identifying the damage-type most harmful to the weed and select an insect doing this type of damage from the literature. 2) Agents are best if from the climatic analogue of the release area, but others can often be adapted. 3) Have patience. Results of spurge control in Spruce Woods Park, Manitoba are discussed. 4) Use consortia for agent distribution and for funding overseas studies. 5) Introduce the agent from the population tested. 6) Lessons relating to the enabling legislation and the release approval process. Problems have reached a crisis point and unless changes are made biocontrol of weeds with native congeneric species, such as leafy spurge, will cease.

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Leafy spurge in Manitoba – The fight to keep ahead

CARLA POUTEAU

Weed Specialist, Manitoba Agriculture.

Approximately 52,000 hectares (130,000 acres) are infested with leafy spurge in Manitoba. Most of the land infested is pasture and rangeland. Since the early 1980's, Manitoba Agriculture has been involved in the biological control of leafy spurge. Biocontrol was investigated after years of herbicide control failures. To date, nine different species of biocontrol agents have been investigated in Manitoba. Agriculture and Agri-Food Canada has been a major partner throughout the project. To date, *Aphthona nigriscutis* and *A. cyparissiae* have been the most successful insects managing spurge in Manitoba. However, these two insects appear quite particular in their habitat. In Manitoba, an insect is needed that will attack spurge in shaded areas (shrub and trees). *Lobesia euphorbiana* is showing some promise. However, this insect only prevents seed set. It does not affect the root system. Most recently (1990's), *Aphthona lacertosa* and *A. czwalinae* have established in Manitoba. Based on the success our neighbors to the south (North Dakota) have had with these two species, Manitoba Agriculture will concentrate on increasing the release sites of *Aphthona lacertosa* and *A. czwalinae* in future years. *Aphthona czwalinae* has proven very hardy in Manitoba as it has survived three weeks of flooding at one location.

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An update of biological control of leafy spurge in Alberta

D. E. COLE¹, K. STROMME¹, and A. S. MCCLAY²

¹Alberta Agriculture, Food and Rural Development Edmonton. ²Alberta Environmental Centre Vegreville.

A co-operative effort between Alberta Agriculture, Food and Rural Development, the Alberta Environmental Centre and Agriculture and Agri-Food Canada and the International Institute of Biological Control in Delemont, Switzerland has seen a large number of biological control agents released in Alberta for the control of leafy spurge. Alec McClay, Alberta Environmental Centre and Peter Harris, Agriculture and Agri-Food Canada, Lethbridge, Alberta have been instrumental in introducing new insect biological control agents into the province for study and monitoring. Once an agent has “proven itself” and there are large numbers available at some of the more successful sites, the Agronomy Unit and Public Land Services of Alberta Agriculture, Food and Rural Development have become involved in the distribution of the biological control agent to suitable sites. The distribution of the most successful agent in Alberta, *Aphthona nigricutis*, has been through “hands-on” redistribution clinics involving the municipal district agricultural fieldmen and producers. Local producers and agricultural fieldmen further redistribute from the more heavily populated agent sites or collection centres in their area.

Leafy spurge is conservatively estimated to infest 15,000 acres in Alberta. There have been over 150,000 *A. nigricutis* released at over 400 sites in Alberta. At one site the leafy spurge biomass was reduced from 172 gm⁻² down to 2 gm⁻² in 5 years with a corresponding increase in grass biomass from 1 gm⁻² up to 57 gm⁻². Only the initial *Aphthona flava* release in Alberta is providing beetles for further redistribution with few of the other 44 releases establishing. As other biological control agents are needed for lower lying, higher moisture and shaded sites, the root-feeding *Aphthona cyparissiae*, *Aphthona czwalinae* and *Aphthona lacertosa* are being tested in Alberta as well as the stem-mining fly *Pegomya* spp., the gall fly *Spurgia esulae*, and the moth *Minoa murinata*.

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Update on Wyoming's leafy spurge research program

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The control of leafy spurge with initial and retreatments of picloram

This research was conducted near Devil's Tower, Wyoming to compare the efficacy of various rates of picloram for leafy spurge control. Plots were retreated to maintain or attain 80% control with light rates of picloram or picloram/2,4-D tankmixes. Initial treatments were 0.25 lb picloram to 2.0 lb picloram in 0.25 lb increments and 0.25 lb picloram + 1.0 lb 2,4-D. Retreatments were 0.25 or 0.5 lb picloram or 0.25 lb picloram + 1.0 lb 2,4-D. The initial treatment of 0.25 lb picloram was retreated only with 0.25 lb picloram and the initial treatment of 0.25 lb picloram + 1.0 lb 2,4-D was retreated only with 0.25 lb picloram + 1.0 lb 2,4-D. Plots were 10 by 27 ft. with four replications arranged in a randomized complete block. The initial herbicide treatments were applied May 24, 1989. Retreatments were applied June 6, 1990; June 13, 1991; June 10, 1992; September 22, 1993; and September 19, 1994. The soil was a silt loam (22% sand, 58% silt, and 20% clay) with 1.8% organic matter and a 6.3 pH. Leafy spurge was in full bloom and 12 to 14 inches in height, for the initial treatments and in full bloom, 12 to 20 inches in height for spring retreatments and 16 to 24 inches in height for fall retreatments. Infestations were heavy throughout the experiment area. Visual weed control evaluations were made June 6, 1990; June 13, 1991; June 10, 1992; June 21, 1993; June 15, 1994; June 27, 1995; and June 18, 1996.

Plots with initial treatments of 1.25 lb picloram or greater in 1989 provided 80% or better leafy spurge control and did not require retreatment in 1990. Initial treatments maintaining 80% control or better in 1991 were 1.5, 1.75 or 2.0 lb picloram treatments. Initial treatments of 2.0 lb picloram were the only treatments maintaining 80% control or better in 1992. The only 1990 retreatment attaining 80% control or better in 1991 was 0.5 lb picloram over an initial 1.0 lb of picloram. None of the retreatments applied in 1991 attained 80% control in 1992. None of the retreatments applied in 1992 attained 80% control in 1993. All 0.5 picloram retreatments applied in the fall of 1993 attained 80% control or better in 1994. One 0.25 picloram + 1.0 2,4-D retreatment applied over an initial treatment of 1.5 picloram attained 80% control in 1994. None of the 2.0 lb picloram treatments have maintained 80% since 1993. No treatments maintained 80% in 1995 and control is declining. Spring retreatments of picloram at 0.25 or 0.5 have not been effec-

tive in attaining or maintaining 80% control. Spring retreatments of 0.25 lb picloram + 1.0 lb 2,4-D appear to be as effective as spring retreatments 0.5 lb picloram. However, spring retreatments of 0.25 lb picloram + 1.0 lb 2,4-D have not attained or maintained 80% control. Fall retreatments of 0.5 lb picloram or 0.25 lb picloram + 1.0 lb 2,4-D may be effective in attaining or maintaining 80% control. The most effective long-term treatment for control of leafy spurge was 2.0 lb picloram.

The control of leafy spurge with imazameth

The objective of this study was to compare the efficacy of imazameth for leafy spurge control. The plots were 10 by 27 feet in a randomized complete block design with four replications. Treatments were imazameth at one, two and 4 oz ai/a with or without a crop oil concentrate and picloram at 0.5 lb ai/a. Treatments were applied with a hand-held CO₂ pressurized six-nozzle sprayer (20" spacing) delivering 20 gpa at 40 psi. Treatments were applied September 26, 1995 and evaluated June 18, 1996. Leafy spurge was mature and 16 to 24 inches tall. The soil was a silt loam with 22% sand, 58% silt, 20% clay; with 1.8% organic matter and Ph 6.3. Depth to parent material is approximately 27 inches.

Imazameth at 4 oz/a plus a crop oil concentrate provided the best control (87%). Without the crop oil concentrate control was only 69%. The addition of a crop oil concentrate greatly improved leafy spurge control. No other treatments provided satisfactory control. There was little or no grass damage when imazameth was applied after grasses were mature in mid September. It appears that imazameth may have potential fit for control of leafy spurge.

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Integrated control of leafy spurge (*Euphorbia esula*) with Bozoisky Russian wildrye (*Psathyrostachys juncea*) and luna pubescent, wheat grass (*Agropyron intermedium* var. *trichophorum*)

M. A. FERRELL, T. D. WHITSON, D. W. KOCH, and A. E. GADE

Extension Pesticide Coordinator, Associate Professor, Professor, Department of Plant, Soil and Insect Sciences, University of Wyoming, Laramie, Wyoming 82071, and Extension Agent, University of Wyoming Cooperative Extension Service, Sundance, Wyoming 82729.

A study was established near Devil's Tower in Crook County, Wyoming to determine the potential of Bozoisky Russian wildrye and Luna pubescent wheatgrass competition as an alternative to repetitive herbicide treatment for control of leafy spurge. Grasses were seeded with or without tillage August 8 1989. Glyphosate was applied before seeding grasses in 1989 to eliminate weed competition with seedling grasses. Applications of 2,4-D and metsulfuron were applied after seeding to control annual weeds. Evaluations made 7 years after seeding have been based on one or more of the following: percent grass stand, plants per 20 feet of row, percent leafy spurge control, percent downy brome infestation, grass yield, and percent canopy cover.

The tilled areas had significantly more plants per 20 feet of row than did the no-till areas for Bozoisky. There was no difference between till and no-till for Luna. The tilled areas had significantly less downy brome than did no-till areas. There is more downy brome in the Bozoisky than the Luna. The downy brome infestation appears to be on the increase. Both grasses had very good yields in the tilled areas and good yields in the no-till areas. Luna has produced more forage than Bozoisky in both the tilled and no-till areas. Luna is maintaining excellent control of leafy spurge in both till and no-till areas (91% in the tilled plots and 86% in the no-till plots). Control in the Bozoisky till plot is at 90% and is 71% in the no-till plots. Percent canopy covers show leafy spurge to be on the increase in both till and no-till plots for both grass species. The increase is greatest in the Bozoisky no-till plots. Both grasses initially had very good establishment in the till and no-till areas and have maintained excellent to fair leafy spurge control 7 years after seeding.

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Niche specificity of insects introduced for leafy spurge control

NEAL R. SPENCER

Biological Control of Weeds, USDA/ARS, P.O. Box 1109, Sidney, Montana 59270.

Leafy Spurge, a noxious perennial weed of Eurasian origin, is a major rangeland problem on the northern Great Plains of the United States and in the prairie provinces of Canada. Because it is an introduced plant, leafy spurge has few native natural enemies. Thirteen host specific insect species have been imported from Europe to the United States for leafy spurge control. Due to the limited occurrence of leafy spurge and closely related species in western Europe and Eurasia, there is limited habitat information for the introduced species. Three flea beetle species, *Aphthona cyparissiae*, *A. flava* and *A. nigriscutis*, were released at multiple sites in eastern Montana and North Dakota between 1990 and 1993. Ecological data and insect numbers were collected subsequent years. Analysis of the data indicates a relationship between leafy spurge plant height and density and the numbers of *Aphthona* species in succeeding generations.

A. nigriscutis is well adapted to the dryer portions of the northern Great Plains. In areas where leafy spurge density is low, plant height under 18 inches and water stress apparent in late summer, *A. nigriscutis* does well. Under these conditions, insect mortality is reduced and leafy spurge control is seen over a limited area within three to five years. As site conditions become more moist, *A. nigriscutis* mortality is increased and spurge control is limited to non-existent. *A. cyparissiae* is difficult to distinguish from *A. nigriscutis*. Similarly, the two species occur in overlapping niches. *A. cyparissiae* will establish in slightly wetter niches than *A. nigriscutis*. Both *A. nigriscutis* and *A. cyparissiae* are well adapted to climatic conditions on the northern plains. *A. flava* remain an enigma. Two sites, in Alberta and Montana, produced large insects numbers and good leafy spurge control. These successful establishments have not been duplicated. There are other *A. flava* sites in Montana and North Dakota with increasing insect numbers. We do not yet understand the characteristics of these sites and how to successfully establish new colonies. Successful insect establishment is important to leafy spurge biological control. Flourishing insect colonies is dependent on a knowledge of insect niche requirements.

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Effect of fall applied picloram and 2,4-D on *Aphthona nigriscutis* population

JEFF A. NELSON, RODNEY G. LYM and CALVIN G. MESSERSMITH

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Aphthona nigriscutis has reduced the density of leafy spurge at many locations. However, there are locations where *A. nigriscutis* has not established or is found at densities too low to be effective. Therefore, it may be necessary to integrate biological and chemical control to reduce leafy spurge to satisfactory levels. The objective of this experiment was to determine the effect of picloram and 2,4-D fall-applied in the field on *A. nigriscutis* population.

Experiments were conducted at two locations, Chaffee and Fort Ransom, North Dakota which average 90 to 63 leafy spurge stems/m², respectively. Approximately 350 *A. nigriscutis* adults were released into 1.8-by 1.8-by 1.8-m cages on June 22, 1995. An additional 100 *A. nigriscutis* adults were released on July 14, 1995. The herbicides picloram plus 2,4-D at 0.56 plus 1.1 kg/ha were applied on four dates, August 15, September 1 and 15 and October 1.

The effect of picloram and 2,4-D on *A. nigriscutis* population was estimated by counting the number of adults emerging from soil cores harvest October 30, 1995 and May 28, 1996 and adults collected in the field in June and July 1996. A golf cup cutter was used to harvest soil cores which were 10.8 cm diameter to a depth of 15 cm, and sample harvested in October were held at 3° C for 75 days. Each sample was then placed into a 2L paper container and covered by a trap chamber, which was a clear plastic cylinder with a mesh top. Trap chambers with soil cores were maintained in the laboratory at 21° C for with a 16-hour photoperiod until *A. nigriscutis* adults emerged. Soil cores harvested in May were placed directly in trap chambers and treated identically to soil cores harvested in October. An insect sweep net was used to collect *A. nigriscutis* from the cage area and portions of the border which totalled 4.5 m².

Leafy spurge density averaged less than 1 stem/0.25 m² on June 5, 1996 regardless of herbicide application date or location. The number of *A. nigriscutis* adults emerging from soil cores obtained in fall and spring was similar regardless of herbicide application date or location. An average of 2 *A. nigriscutis* adults were recovered from each soil core harvested in the fall compared to only 1 per core from spring harvested samples, which indicates overwintering mortality. Peak field emergence of *A. nigriscutis* adults averaged 33/4.5 m² on July 10 at Chaffee and 7/4.5 m² on July 18 at Ft. Ransom. The number of *A. nigriscutis* collected in the field was similar regardless of herbicide application date at each location.

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Manitoba Weed Supervisor's Association past, present and future?

RUBY REIMER

Southeast Weed Control District.

- The development of Manitoba Weed Districts and specifically the role of Manitoba Weed Supervisors from the inception of the Weed District program to the present.
- Status of Weed Districts and Supervisors - Then and Now.
- The relationship between Manitoba Agriculture and the Manitoba Weed Supervisor's Association and how it has changes in recent years in the areas of Program Planning, Funding and Training.
- Manitoba Weed Supervisor's Association special projects (eg. Bio-control, Weed Resistance, Purple Loosestrife, etc).
- The evolution of the Manitoba Weed Supervisor's Association as it has worked to become self-driven and how the Association and Weed Districts have coped with funding problems. Looking to the future.

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1996 Weed supervisors and the new utility

ERIC RICHARDSON

R. M. of Dauphin

Using a holistic approach to controlling noxious weeds, we have incorporated the use of insects at bio-release sites.

The guide post to a site's success requires semi-annual avoits at the first point of release.

A GPS receiver lends itself cheaply and accurately to this end; within minutes a GPS can establish the position of a release site. The same point can be re-established repeatedly so bio-sites can be precisely monitored.

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Phenology maps for leafy spurge biological control agents: Development and application in the U.S.

RICH HANSEN

USDA-APHIS- PPQ, Forestry Sciences Lab, Montana State, University, Bozeman, MT 59717-0278, USA.

Five species of *Aphthona* flea beetles (Coleoptera: Chrysomelidae) and the gall midge *Spurgia esulae* (Diptera: Cecidomyiidae) are among the insects introduced into the U.S. as classical biological control agents of leafy spurge (*Euphorbia esula*). Nonlinear models describing the seasonal occurrence of adult *Aphthona* spp. and *S. esulae* life stages were developed using data from two Montana sites (1993-1995) and one Montana site (1992-1995), respectively. Models for all species exhibited r^2 values exceeding 0.60. Peak occurrence of adults of *A. nigriscutis*, *A. czwalinae*/*A. lacertosa* (mixed populations), *A. cyparissiae*, and *A. flava* occurred at 1230, 1320, 1565 and 1580 degree-days accumulated from January 1, respectively ($>0^\circ\text{C}$). The occurrence first-generation *S. esulae* pupae (the optimal life stage for collection) peaked at 990 accumulated degree-days $>0^\circ\text{C}$. The mean calendar dates by which these degree-day values accumulated were calculated for nearly 300 weather stations in the central and western U.S., using temperature data sets that spanned 40 to 110 years. These data were then used to recommend sampling dates (i.e. scheduled to anticipated peak adult abundance) in the various U.S. states. Contour maps were prepared that displayed predicted *Aphthona* spp. sampling or collection dates in the western and midwestern U.S. (based on anticipated peak adult abundance), over weekly intervals ranging from May 15 to August 28 depending on species and location. A similar contour map was prepared for *S. esulae* collections, based on peak first-generation pupae; weekly intervals ranged from April 24 to July 17. Phenological events generally happened earliest in New Mexico, southern Utah and southern Colorado and latest in higher-elevation portions of western Wyoming and eastern Idaho. Additional applications for phenology maps include determining relationships between agent populations and length of growing season, and the general determination of “bioclimatic suitability” of different areas of the U.S. for a given agent.

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Department of National Defence's involvement in leafy spurge management to date at Canadian Forces Base Shilo, Manitoba, Canada

GARNET SHEARER and SHERRY LYNN PUNAK

Base Environment Office, Base Construction Engineering, CFB Shilo, Manitoba, R0K 2A0.

Control of the noxious weed, leafy spurge, has been steadily increasing since the mid 1980's at CFB Shilo training ranges. The use of chemical control has changed significantly during the last decade. Currently only the outer areas of the training ranges are sprayed with 2,4-D Amine. Biological control began at Shilo in 1986 with test plots for biological agents placed in various locations throughout the training ranges. DND has, in the past, actively participated in the research in bringing new biological agents for release by providing monetary support and research data. Research efforts at Shilo are controlled by the weather and access to the training area.

Data sheets listing the topography and vegetation at release sites have been completed and filed with pictures of the release sites. To date, CFB Shilo now has 196 biological release sites throughout the ranges. Concentration of release in the last two years has been in the southern portion of the ranges to prevent the spread of spurge into native mixed-grass prairies which dominate the southern portions. This year, the Base Environment Office will be determining a Standing Operating Procedure for the releasing and monitoring of biocontrol sites based on data collected during the last seven field seasons.

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Leafy spurge control on Canada's largest tall grass prairie preserve

GENE FORTNEY

Manitoba Director, Nature Conservancy, 298 Garry Street, Winnipeg, Manitoba R3C 1H3.

In the late 1980's the Manitoba Naturalists Society discovered remnants of native tall grass prairie in south-eastern Manitoba. This discovery has led to the development of the largest tall grass prairie preserve in Canada and one of the largest in North America. The Manitoba Tall Grass Prairie Preserve has progressed through the initial survey stage into an era of prairie management. Originally settled by agriculturalists in the late 1880's, the land was too stoney and poorly drained for intensive agriculture. The land was used for pasture and hayland for many years and finally abandoned. Invasive native species such as aspen, willow and several other woody species as well as the introduction of exotic grasses and forbs have made management a formidable challenge. The discovery of noxious weeds such as Leafy Spurge and St. John's Wort has exasperated the management problem. An integrated vegetation management system has been adopted to combat these challenges. The effectiveness of limited herbicide use, mechanical removal and the use of biocontrol agents such as *Lobesia euphorbiana*, *Aphathona cyparissiae* and *A. nigriscutis* are currently being monitored on the control of Leafy Spurge while other options are being considered.

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Imazameth is safe on grass, leafy spurge is becoming resistant to picloram and other myths?

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Imazameth (AC 263,222) has been shown to provide good leafy spurge control with acceptable grass tolerance for warm-season species in Nebraska. However, cool-season grass injury was severe when imazameth was evaluated in North Dakota. Leafy spurge control in June 1996 increased as application rate increased and averaged 80 to 100% when imazameth was applied from 0.125 to 0.5 lb/A, respectively, on September 18, 1995. Unfortunately, grass injury to cool-season species ranged from 10 to 65%. Imazameth is currently being evaluated at lower rates, alone and with additives, and as a spring applied treatment in an effort to obtain good leafy spurge control with minimal grass injury in North Dakota.

Glyphosate plus 2,4-D plus 0.6 lb/A will provide 70 to 90% leafy spurge control after one treatment but can cause severe grass injury with repeated applications. A series of experiments was established at three locations to compare cost and efficacy of glyphosate plus 2,4-D as part of a long-term management program for leafy spurge control. The initial treatments of glyphosate plus 2,4-D or picloram plus 2,4-D were applied in late June of 1993 and were retreated with the same or an alternate treatment in 1994 and 1995. Visual evaluation were taken annually from 1993 to 1996. Glyphosate plus 2,4-D provided 75% leafy spurge control 12 months after treatment (MAT) compared to 30% for picloram plus 2,4-D. All treatments provided similar control when annually applied for 3 years but the total treatment cost was variable. Glyphosate plus 2,4-D applied three consecutive years provided 73% control with only 10% grass injury and cost \$27/A. Glyphosate plus 2,4-D applied in 1993 and 1995 with picloram plus 2,4-D applied in 1994 averaged 80% control and cost \$31/A. Three annual applications of picloram plus 2,4-D provided 70% control and cost \$40/A. There was no significant grass injury for any treatment.

Picloram is one of the most effective herbicides for leafy spurge control. Previous research at North Dakota State University has shown that picloram plus 2,4-D at 0.25 plus 1 lb/A will provide approximately 85% control or better after 3 to 5 years of annual treatment. Picloram alone at 1 to 2 lb/A will provide acceptable leafy spurge control for

18 to 24 months in North Dakota. Recently at field tours and educational meetings land managers have expressed concern that picloram provides less leafy spurge control than they have come to expect. To determine if leafy spurge was becoming resistant or tolerant to picloram, the average leafy spurge control with picloram and picloram plus 2,4-D treatments applied from 1963 to 1982 (historical) was compared to the same treatments applied from 1983 to 1985 (present). The average control was less with present day treatments for every picloram treatment regardless of application rate, if applied alone or with 2,4-D, or in the spring or fall. For example, picloram at 0.5 lb/A alone historically averaged 85% control 12 months after treatment compared to an average of 55% control with the present day treatments. Also, picloram at 1 lb/A provided 88 and 68% control when the historical average was compared to the present treatment average, respectively.

The reason for the better control observed with the historical compared to present treatments may be due to the plant becoming resistant to picloram, or the more susceptible plants have been controlled and only the most vigorous plants remain, or the personnel conducting the evaluation have become more demanding. To determine if control indeed was decreasing with time, the average control from 1984 to 1988 was compared to the average control of the same treatments applied from 1991 to 1995. The same personnel conducted the evaluations in both time periods. The average control was similar to slightly better from 1991 to 1995 compared to treatments applied from 1984 to 1988. If leafy spurge control with picloram was declining with time, the control observed from 1984 to 1988 should have been better than that from 1991 to 1995. Thus, it is not likely leafy spurge has become resistant to picloram, or that only the most vigorous or tolerant plants remain. It is likely the expectations of control with picloram have increased and historical evaluators tended to rate control higher than present.

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Leafy spurge control with sheep

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Leafy spurge, a perennial weed, continues to invade hundreds of thousands of acres across Manitoba. Previous attempts to control spurge using cultivation or herbicides have been largely unsuccessful. In 1993, the Brandon Soil Management Association initiated a project to determine the impact sheep grazing would have on the density and longevity of established spurge infestations. Results to date are very encouraging. Spurge density is recorded each spring to measure the degree of control obtained from the previous grazing seasons. Observations in the spring of 1995 showed the following reductions in spurge density after only two years: paddocks sprayed with 2,4-D only, 44%; paddocks grazed by sheep only, 54%; paddocks sprayed with 2,4-D and grazed by sheep, 61%. Observations of pasture conditions during the 1995 grazing season suggest further control can be expected next spring. Sheep performance has been good in all three grazing seasons. Dry ewes gained an average of 0.167 lb/day in the 1993 and 1994 grazing seasons. In 1995, sheep gains improved to 0.275 lb/day in the sheep only paddocks and to 0.295 lb/day in the sheep plus 2,4-D treated paddocks. In conjunction with this trial, three additional projects are being run. In 1994, a stocking rate trial was initiated to determine how spurge control is related to sheep stocking rates. As in 1995, the Manitoba Sheep Association ran two trials to determine if the undesirable compounds found in spurge could be found in the sheep's blood and to find out if grazing spurge causes off-flavours in meat of animals grazing spurge.

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A simple technique for separating *Aphthona* flea beetles from debris

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USDA, APHIS, PPQ in North Dakota utilizes the volumetric method for counting *Aphthona* flea beetles as developed by the APHIS Bozeman Biocontrol lab. We have found it to be an efficient and practical in-the-field technique for counting large numbers of flea beetles. The purpose of our efforts is to provide flea beetles from insectaries to county weed boards or other land managers. Field crews collecting flea beetles from insectaries often get more plant debris and other insects than flea beetles. The debris makes it difficult, if not impossible, to use the volumetric counting technique.

The process of counting the beetles for redistribution is faster and easier by using this sorting technique devised this season. The sorting and counting is done in conjunction with an assembly line system of packaging beetles. We have utilized this technique at an excellent site to package approximately 2 million beetles, one thousand beetles per package, in one day. Weed board officials that are distributing beetles to land managers may find the technique useful.

The sorting system uses plastic pipe, 4 inches in diameter and 6 inches long, that is perforated using a 3/16th inch drill bit. To save weight, we use pipe rated only for vent and drain. It is thinner walled than pipe rated for water lines. Plastic end caps for this pipe are used and also perforated. One cap was glued onto the perforated pipe. We use white pipe to reflect heat, however, the inside of the pipe is sprayed with black paint so that the only light entering the pipe was through the holes.

The premise of this tool takes advantage of the flea beetles extreme attraction to light. The tubes are filled approximately 1/2 full of the beetles and debris. Six of the containers are placed in an 18 by 24-inch white nylon bag with a drawstring. The bag is placed in direct sun and within several minutes, the beetles have escaped the perforated containers and are trapped in the bag. The plastic containers then contain only plant debris, grasshoppers and other insects. The volumetric counting method can then be used with greater ease and accuracy. Nylon or muslin bags are recommended over mesh bags because the beetles can cling better to mesh.

We have experimented somewhat with the size and number of holes in the containers. Generally, we have found that you cannot have too many holes but if the holes are too large, other insects and small debris begin to come out with the beetles.

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***Aphthona* spp. flea beetle movement along railroad right-of-ways**

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Leafy spurge is often found in long narrow corridors such as railroad right-of-ways where it is difficult to treat. Two experiments were conducted to determine the establishment, population increase, and movement of *Aphthona* species flea beetles on a railroad right-of-way.

A. nigriscutis was released on June 28, 1993 in a dense stand of leafy spurge along a 2.5 mile stretch of the Burlington Northern railroad right-of-way near Buffalo, ND. There were five treatments consisting of 100, 200, 300, 400 and 500 adult insects distributed per release point. Release points were 260 feet apart and replicated three times. Stem density and adult flea beetle population were monitored in the spring and summer, respectively, at the release point and at distances 10, 25 and 40 feet in a semi-circle pattern from the release point.

A. nigriscutis flea beetles were found in all treatments each year after release and leafy spurge stem density began to decline in 1995. The stem density decreased from an average of 18 stem/0.25 m² in 1993 to 7 stems/0.25 m² in 1996. The greatest stem density decrease was 72% when 500 beetles/treatment were released. This decrease occurred 10 feet from the release point for all treatments where beetle populations were the highest. *A. nigriscutis* population in the 300 and 400 insects/release point treatments averaged 8 beetles/m² compared to 2 beetles/m² for all other treatments.

A similar experiment was established on July 10, 1995 with *A. czwalinae* along the Red River Valley and Western railroad right-of-way near Lisbon, ND. The number of insects used was increased to 500, 1000, 1500 and 2000 adults per treatment. Release points were 150 feet apart and replicated four times. Stem density and adult flea beetle population were monitored in the spring and summer, respectively, at the release point and at distances of 10, 30 and 50 feet in a circular pattern around the release point.

A. czwalinae were found at all release sites in 1996. The average stem density in the 2000 insects/release point declined from 21 stems/m² to 15 stems/m² 1 year following release while stem density in all other treatments was unchanged. Flea beetles will establish on industrial sites such as railroad right-of-ways. The larger the number of insects released the more rapid the leafy spurge stem density declined.

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2,4-D reversal of DFMO-induced inhibition of adventitious root formation in leafy spurge (*Euphorbia esula* L.) in vitro

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Indoleacetic acid (IAA) stimulates adventitious root formation and partially reverses the effects of difluoromethylornithine (DFMO), a specific suicide inhibitor of putrescine biosynthesis, in etiolated aseptically grown hypocotyl segments of the perennial weed, leafy spurge. DFMO also is a strong inhibitor of adventitious root formation in the hypocotyl segments. Dichlorophenoxyacetic acid (2,4-D) has auxin-like activity and was also tested to determine if it could counteract DFMO-induced inhibition of root formation. When 2,4-D was applied at 45 to 450 nM in the nutrient medium, root formation was inhibited. Unexpectedly, 450 nM 2,4-D applied simultaneously with 500 pM DFMO reversed the DFMO-induced inhibition of adventitious root formation, forming root numbers similar to controls. This reversal occurred under growth conditions with complete B5 nutrient medium or with B5 medium containing salts and vitamins reduced to 10% of their normal concentrations. This action of 2,4-D resembles that of indoleacetic acid (IAA), although 2,4-D did not stimulate root formation in the absence of DFMO, as did IAA (at 230 to 1100 nM).

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Cloning and expression of a cold-regulated gene from leafy spurge (*Euphorbia esula* L.)

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Leafy spurge (*Euphorbia esula*) is a perennial weed which is capable of acclimating to sub-freezing temperatures. We have used the differential display technique to identify and clone a cDNA for a cold regulated gene (*Cor103*) whose mRNA accumulates specifically during the cold acclimation process. The expression of *Cor103* was analyzed, and it has been determined that the RNA from this gene reaches maximal expression in less than 2 days following exposure of the plant to temperatures of 5°C, and remains at high levels in the plant for at least 30 days so long as the plant is left in the cold. RNA from *Cor103* return to control levels within 24 hours when the plant is returned to normal growing temperatures. *Cor103* transcript does not accumulate under conditions of drought or heat stress. The *Cor103* gene is induced in response to low temperatures in roots, stems and leaves, but is expressed at high levels in tissue culture at control temperatures. Southern blot analysis indicates that the *Cor103* gene is a member of a gene family. Sequence analysis indicates that the 3' end of the *Cor103* gene is extremely rich in glycine, and thus shares some homology to a group of glycine-rich RNA binding proteins, some of which have also been shown to be cold regulated.

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Cloning and characterization of a genomic clone of the root bud growth-response gene Gro7 from leafy spurge (*Euphorbia esula* L.)

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One of the key features of leafy spurge is the presence of a large number of below-ground shoot meristems located along the roots of the plant (referred to as root buds) which remain dormant until they are separated from the aerial portion of the plant. Previous results indicated that loss of contact to the aerial plant parts results in changes in gene expression in the cells of the root buds. We have reported cloning of a partial cDNA for one of the genes, Gro7. Here, we report the cloning of a partial genomic clone of the Gro7 gene. A genomic library (of 2×10^6 primary pfu with an average insert size of ~6 kilobases) was constructed. This library was screened by hybridization to the Gro7 cDNA clone. Eight out of 500,000 plaques hybridized to the cDNA. Since the Gro7 gene is a single copy gene, this result indicates that the leafy spurge genome is roughly 3.75×10^8 bp/haploid genome. One of the genomic clones was further purified and characterized by restriction mapping. This clone is currently being sequenced in hopes of gaining information concerning the function of this gene.

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Differential tolerance toward oxidative stress between immature and mature leafy spurge leaves – An evolutionary mechanism for survival under adverse environmental conditions?

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The most effective method for controlling leafy spurge is to kill the crown and root buds or to break bud dormancy and kill the newly emerging shoots. However, crown and root buds did not break dormancy and emerge unless the entire shoot or above-ground portion of the plant was excised or killed with 2,4-D. The apical leaves are able to survive severe conditions such as moisture stress, pathogen invasion (mildew), herbicide (diclofop-methyl), etc., whereas more mature leaves were killed rapidly and abscised. The various dissimilar stresses mentioned above are known to have a common lethal mechanism, i.e. the induction of active oxygen species (AOS)(free radicals) to cause rapid cell destruction and death. A consequence of AOS formation is the secondary induction of ethylene which is not the lethal factor. Diclofop-methyl (DM) was used as a physiological probe to induce oxidative stress on leafy spurge. Significant increases in ethylene was induced by DM and 2,4-D over control tissues in mature and immature leafy spurge leaves. However, only mature leaves were killed by DM whereas all leaves were killed by 2,4-D. AVG (aminoethoxyvinyl-glycine) inhibited completely ethylene induction by both 2,4-D and DM. Conversely, vitamin E (tocopherol) inhibited DM induction of ethylene by approximately 50% of control but it had no effect on ethylene induction by 2,4-D. Therefore, the action of the inhibitors on ethylene production indicates that DM induces death of mature tissues by a free radical oxidation mechanism in contrast to 2,4-D which induces ethylene by an alternative mechanism not involving AOS. Tocopherol, an effective scavenger of AOS, protected leafy spurge hypocotyls from damage by DM in tissue culture. It is hypothesized that apical tissues may survive oxidative stress due to more active antioxidation systems than mature tissues and, therefore, prevent root buds from emergence and damage under adverse environmental stress conditions.

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Reproductive status of established black dot flea beetle (*Aphthona nigriscutis*) populations in South and North Dakota

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Black dot flea beetles, *Aphthona nigriscutis* Foudras, are used for biological suppression of leafy spurge, *Euphorbia esula* L. Thousands of adults are collected annually from insectaries in several states for redistribution to other spurge infested areas. However, little is known about the reproduction biology of black dot flea beetles. The purpose of this research was to monitor the reproductive status of black dot flea beetles under field conditions. In 1995, adults were collected weekly from an insectary near Pollock, SD by sweep net sampling from June 26 through August, when flea beetle adults were no longer present in the samples. One hundred adults were examined and gender ratios determined. The spermatheca of 25 females were removed from the abdomen and analyzed for the presence of sperm. Oocyte maturation in these females also was determined by rating oocyte development. Males were rare in sweep net samples and did not exceed 4% of the adult population throughout the summer. The insemination rate of females on July 6 was 44% and was significantly ($P < 0.01$) higher than previous or subsequent sampling periods. Following July 18, only about 20% of adult females from samples were inseminated. Oocyte maturation gradually increased to a peak on July 25. Adults were rare at the Pollock site on August 3 and were not present on August 22.

In 1996, the study was expanded to include two North Dakota populations (Theodore Roosevelt National Park, Valley City). Adults began to emerge at the three sites between June 14 to 26. The percentage of males in the Pollock, Valley City and Theodore Roosevelt National Park samples (from the first week) was 6.9%, 14% and 20%, respectively. Analysis of weekly samples is continuing and additional results will be reported. These data are useful in determining the optimum period for collection of reproductively viable individuals. These data could also be used as an index for numbers of individuals needed to be released in redistribution sites.

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Biocontrol of leafy spurge with *Aphthona nigriscutis* in Alberta “The Beverly Bridge Site”

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There are over 130 research sites in Alberta where *Aphthona nigriscutis* beetles have been released on leafy spurge. Once beetles have established, the population is monitored and vegetation data is collected from the sites. *A. nigriscutis*, the black dot spurge beetle, is most effective on lighter soils with leafy spurge flowering heights of less than 70 cm, and on high, dry slopes or hilltops.

The Beverly Bridge in Edmonton is one of the most successful *A. nigriscutis* sites in Alberta. In 1989, 500 beetles were released at one of six sites on the Beverly bridge. The site is located on a dry, south-facing railway embankment. The soil texture is 82% sand, 14% silt and 4% clay. The leafy spurge was very dense at 279 shoots/m², and the above-ground leafy spurge biomass at the time of the release was over 170 g/m². The flowering leafy spurge canopy was 60 cm in height.

In 1992, the beetle population peaked, and by 1993 the leafy spurge biomass was reduced to less than 2 g/m², while the grass biomass increased to 43 g/m². The leafy spurge density and percent cover were also dramatically reduced after four years time.

Year	Leafy Spurge % cover	Leafy Spurge Shoots/m ²	Leafy Spurge Biomass g/m ²	Grass Biomass g/m ²
	40	279	172.6	1.4
	25	395	140.1	18.9
	6.6	109	26.7	14.1
	1	8	1.6	42.9
	1.5	8	2.2	57.1
	1.7	5	1.7	43.3

Beverly Bridge Site # AB-89-0015

There have been over 100,000 beetles collected and redistributed from this site.